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Laboratory of Genetics

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The Laboratory of Genetics was established in Autumn, 1997 by David Schlessinger, with a Human Genetics Unit, a Transcription Remodeling and Regulation Unit initiated by Weidong Wang, and a Gene Recovery and Analysis Unit headed by Ramaiah Nagaraja. A fourth unit, the Developmental Genomics Section, is programmed to start in mid-1998.

The interests of the Laboratory are based on the view that aging has genetic determinants as an integrated part of human development, with a profound dependence on the interplay of synthetic and degradative processes that are initiated in utero. Three major types of study are projected:

1. Transitions between immortal and mortal cells, particularly at the level of large-scale regulatory phenomena at the level of chromatin. For example, the transition of immortal embryonic stem cells to mortal differentiating cells is a fundamental feature of the initiation of aging in metazoans. The genes specifically activated and repressed during such transitions are being studied in mice, both by differential assays of gene expression in 3.5 days post coitum (dpc) mouse embryos and by the analysis of differential function of mutant and unmutated helicases that are affected in premature aging syndromes (the latter in the Unit on Transcription Remodeling and Regulation).

2. Cohorts of genes involved in the development of selected “nonrenewable” systems. To understand and ultimately try to compensate for loss of cells and tissues during aging, the examples of skin appendage and pronephros-kidney development are being studied. Studies start from human or mouse hereditary defects that have been attributed to single genes, such as the ectodysplasin-A involved in X-linked ectodermal dysplasia or the emx2 gene required for kidney formation.

3. Genes involved in embryonic events that prefigure aging-related phenomena. For example, the Human Genetics Unit is involved in studies of overgrowth syndromes, in which the set point of size of tissues and organs is determined in fetal life; and in studies of premature ovarian failure, in which the aging phenomenon of early menopause is determined by an increased rate of follicular atresia during fetal life.

The laboratory is equipped with state-of-the-art resources for genomic approaches in the Gene Recovery and Analysis Unit, including large-insert clones and recovery methods, high throughput sequencing, nuclear fractionation and chromatin analysis techniques, and the potential to make and analyze high-quality cDNA libraries from very few cells from subregions of embryos. It also benefits from collaborative efforts with other groups and resource providers both within NIA and at a number of extramural sites in the United States and abroad.

Laboratory of Genetics Staff

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X chromosome
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Recent Publications:

Pilia G, et al. *Nature Genetics* 1996; 12: 241-247.

Kere J, et al. *Nature Genetics* 1996; 13: 379-380.

Nagaraja R, et al. *Genome Research* 1997; 7: 210-222.

Srivastava AK, et al. *PNAS* 1997; 94: 13069-13074.

Biography: Dr. Schlessinger received his Ph.D. from Harvard University in 1960. Following postdoctoral training at the Pasteur Institute in Paris, he joined Washington University in St. Louis, where he served as Professor of Molecular Microbiology, Genetics, and Microbiology in Medicine until his move to NIA in September, 1997. He has contributed both to microbial and human genome studies. He has served as President of the American Society for Microbiology in 1995, and as the Director of the Human Genome Center at Washington University from 1987-97. During his tenure as Center director, he oversaw the development of the X chromosome map and of much related technology, with the concomitant finding of a number of disease genes. He is currently a councillor of the Human Genome Organization.

Human Genetics Unit: The program is designed to complement studies by many groups in lower animal models and in fibroblast senescence with corresponding studies of embryonic events critical for the aging of specialized mammalian cells and concomitant aging-related phenomena.

1. Studies at the level of gene regulation in chromatin. Projects are designed to understand tissue- and developmentally-restricted expression of the genes in which mutation causes the inherited conditions SGBS or EDA (see below). Promoter and enhancer element function will be analyzed in those instances and in another in which a gene (SYBL1) is expressed on X but not on the Y homologue; it may be repressed by nearby Y heterochromatin. The regulatory processes in all these cases involve features of chromatin; analyses of open and closed chromatin are projected for the genes recovered in chromatin form in artificial chromosomes.

2. Cohorts of genes involved in selected processes, using a “genome approach” to developmental phenomena. The approach starts from human inherited conditions and relevant embryological studies in mouse models (where sets of genes from embryonic stages can be easily mapped in the

genome and localized in sections, and knockout technologies are available). Examples include:

Premature ovarian failure. A set of translocation breakpoints in a “critical region of the X chromosome” are associated with POF. We are analyzing the breakpoints to look for genes or structural features in the chromosomal DNA that can limit ovarian function. In correlated developmental work, systematic studies are beginning of gene cohorts specifically expressed during the development of the kidney and urogenital tract, including ovary and testis.

Hypophosphatemic rickets, X-linked. The responsible gene has been sequenced. It encodes a putative endopeptidase (with an as-yet unknown substrate), and is expressed along the kidney-urogenital developmental axis and in bone precursors. The gene and its protein would be investigated developmentally and biochemically; the HYP mouse has been shown to be an expermental model for the human disease.

Simpson-Golabi-Behmel syndrome (SGBS). Gigantism and overgrowth, particularly of mesoderm-derived tissues and organs, results from mutational lesions in a matrix glycoprotein, glypican 3. The speculative model for the etiology of the disease sees the determination of the set point for organ size as based on IGF2 and related features of growth hormone action. Tests and extensions of this hypothesis are based on developmental studies, including the generation of a mouse model.

X-linked anhidrotic ectodermal dysplasia (EDA). The gene provides an entree to an embyonic branch point that leads to teeth, hair follicles, and sweat glands. The Tabby mouse has been shown to be an experimental model for the human condition, and interacting genes can be found both by genomic approaches and by genetic studies of some of the other 150 inherited ectodermal dysplasias.

The projected work will depend on the Gene Recovery and Analysis Unit and collaborating groups, both for the developmental analysis of gene cohorts and for studies of physiology in aging populations with the aim of facilitating long-term patient benefit.

Collaborators: Professor J.M. Cantu, University of Guadalajara Medical School; Dr. Michele D’Urso, International Institute of Genetics and Biophysics, Naples; Professor Raj Thakker, M.D., Royal Postgraduate Medical School, London; Professor Antonino Forabosco, University of Modena; Dr. Giuseppe Pilia, Italian Research Council, Cagliari; Dr. Juha Kere, University of Helsinki; Dr. Anand Srivastava, Greenwood Genetics Center.

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Recent Publications:

Wang W, et al, *Genes & Dev* 1996; 10: 2117-30.

Wang W, et al, *Embo J* 1996; 15: 5370-82.

Wang W, et al. *Proc Nat Acad Sci* 1998; 95: 492-498.

Biography: Dr. Wang was trained as a biochemist and a molecular biologist at both UCLA, where he obtained his Ph.D., and Stanford University, where he worked as a postdoctoral fellow. His research has focused on the regulation of mammalian gene expression at the chromatin level. He has purified to homogeneity one of the first ATP-dependent chromatin-remodeling complexes in mammals, and has subsequently cloned all the subunits within one complex. His current projects include purification of novel tissue-specific chromatin-remodeling complexes, and investigation of how these complexes are involved in development and differentiation.

Structural and Functional Studies of Mammalian SWI/SNF-Related Chromatin-Remodeling Complexes: The establishment and maintenance of transcriptionally active and inactive chromatin structure in higher eucaryotes play a key role in gene regulation during development, differentiation and adaptation to environmental stimuli. Evidence accumulated during the last two decades indicates that chromatin structures are remodeled when multipotent precursor cells develop into terminally-differentiated cells. However, the underlying mechanism of chromatin remodeling is poorly understood, primarily because molecules that remodel chromatin structures have been discovered only recently. Several molecules identified so far are all multisubunit complexes, but little is known about their structures, functions and mechanism of action. It is not even clear how many chromatin remodeling complexes exist in any given species. The investigation of the chromatin remodeling process will be an exciting field for the next decade.

My research project has focused on one type of chromatin remodeling complex. The mammalian version of SWI/SNF complex. The SWI/SNF complex, originally identified in yeast, functions as a chromatin remodeling machine in signaling pathways that lead to activation of gene expression. The complex was also reported as a part of yeast RNA polymerase II holoenzyme. In Drosophila the complex is required for

control of important developmental regulators, such as homeotic genes and segmentation genes. In mammals, the SWI/SNF-related complexes appear to be involved not only in gene regulation, but also in targeting of HIV integration, in tumor suppression by interacting with RB protein, and in T cell leukemia. It was shown recently that one complex is essential for viability of a mouse embryonal carcinoma cell line. I have completely purified not one, but several distinct mammalian SWI/SNF-related complexes. By microsequencing, my colleagues and I have cloned all 10 subunits from the major complex of human KB cells. Six of them belong to five different multigene families. In one case, three members of the same gene family have different tissue expression patterns, suggesting the existence of tissue-specific chromatin remodeling complexes. Cloning of these new subunits and their family members has paved the way for future investigation of the roles of chromatin-remodeling complexes in development, differentiation and other biological processes in mammals.

My research project is directly related to several human diseases, including aging-related diseases such as Werner's syndrome. At least one subunit of the human SWI/SNF complex belongs to a huge family of ATP-dependent helicases. About half of the human helicases discovered to date are related to human diseases, which include the Werner's Syndrome gene (WRN), Cockayne's Syndrome (ERCC6), Xermaderma pigmentosum, Bloom's Syndrome and ATR-X (X-linked mental retardation with L-thalassemia) Syndrome. Many of the gene products have only been identified recently and their mechanism of action are not known. Studies of SWI/SNF-related complexes provide a way to approach the analysis of function of these helicases, and can facilitate clinical research on pathological aspects of these diseases.

Collaborators: Dr. Gerald Crabtree, Stanford University; Dr. Robert Tjian, University of California, Berkeley; Dr. Matthew Scott, Stanford University; Dr. Jerry Workman, Penn State University; Dr. John Tamkun, University of California, Santa Cruz; Dr. Jacques Cote, Laval University Cancer Research Center; Dr. Bradley Cairns, Harvard Medical School; Dr. Kristine Swiderek, City of Hope Hospital, Los Angeles; Dr. Terry Magnuson, Case Western Reserve University; Dr. Michael Carey, University of California, Los Angeles.